STUDIES OF SOME GLOBULINS AND DEAMIDIZED PROTEINS

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SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA

BY

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L. C.

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
JUNE 1927

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PHYSICO-CHEMICAL STUDIES ON PROTEINS. IV

A Comparative Study of the Acid and Alkali Binding of Native and Deaminized Proteins*

BY WILLIAM MARTIN SANDSTROM

I. Introduction

In order to make a comparative study of the acid- and base-binding capacities of a variety of proteins it was thought desirable in this laboratory to prepare several representative members of each class under as uniform conditions as possible and to submit them to chemical analysis together with the physico-chemical measurements. The first paper in this series (Hoffman and Gortner (17)) dealt with twelve prolamines from the wheat and the maize groups, with casein and fibrin for purposes of comparison. This paper represents a similar study of some typical globulins and several deaminized proteins.

II. Historical

The binding of acid and alkali by proteins. It has been known for the past sixty years that the proteins are amphoteric. During that period many measurements have been made of their capacity to take up acid or alkali from solution. The literature is too voluminous to review in detail here but excellent summaries are given by Robertson (42), Cohn (5), and Hoffman and Gortner (17).

The earlier work was done with such chemical means as were available. Proteins were titrated with dilute acid or alkali to neutrality as determined by indicators. This method has two disadvantages: the end-points are not very distinct and only the binding at the hydrogen ion concentration of the indicator change can be measured. Thus one gram of casein neutralized 55×10^{-5} equivalents of alkali using litmus as an indicator (60) but required 90×10^{-5} equivalents with phenolphthalein.

Attempts were also made to precipitate or coagulate the "protein salts" with alcohol or other precipitants. However, it has since been shown by Robertson (46) that the coagulation of the alkaline caseinates through the addition of alcohol to the system is preceded by a decrease in the degree of dissociation of the protein complex. He also pointed out (Robertson (45)), that by using the potentiometric method (a static method) twice as much alkali was bound per gram of casein as that calculated by Spiro and Pemsel (55) from experiments where ammonium sulfate was used to precipitate the sodium caseinate.

* Published with the approval of the Director as Paper No. 858, Journal Series, Minnesota Agricultural Experiment Station. This paper is a report of work carried out under the Minnesota Agricultural Experiment Station Project on "The Chemistry of the Proteins" under the leadership of Ross Aiken Gortner. Division of Agricultural Biochemistry, Minnesota Agricultural Experiment Station.

Solubility methods have also been used. Some of the older work reports the combination in terms of the amount of acid or alkali bound when the acid or alkali is "saturated" with the protein. Such values are obtained when an excess of the insoluble protein is added to the aqueous system. Thus Robertson, (45) found that one gram of casein required 32×10^{-5} equivalents of hydrochloric acid, and 11.5×10^{-5} of alkali. Hoffman and Gortner (17) list over thirty values obtained by various workers on the more common proteins, but the values reported from various laboratories do not agree very well.

More reliable information has been obtained from physicochemical studies because the measurements depend upon static methods and a wider range of hydrogen ion concentrations can be included. The cryoscopic method has been used by Bugarzsky and Liebermann (4), and Takeda (56), but it is to be recalled that such values were obtained at about 0°C. and that the temperature coefficients are of considerable magnitude especially at higher concentrations of acid and alkali (17). Bugarzsky and Liebermann (4) found that 6.4 grams of egg albumin added to 100 cubic centimeters of 0.05 normal hydrochloric acid or sodium hydroxide decreased the value of Δ by 50 per cent indicating that the total number of ions and molecules in the system had been decreased one half. They could detect no effect of their protein on neutral salt solutions. The freezing point method has not been widely used, probably because it is not sufficiently sensitive and has been supplanted by electrical methods. The same may be said for the measurements of the concentration of hydrogen ions by their catalytic effect on the rate of inversion of sucrose.

Measurements of electrical conductivity have been made on acid or alkali solutions to which proteins were added. The method depends upon the fact that protein salts are apparently less highly ionized than are the equivalent concentrations of acids and alkalis, and that the ions have a low migration velocity because of their mass and volume. Hardy (10) used this method, as did Mellanby (31) and Robertson (47).

Probably the potentiometric method has been most widely used. A large number of measurements have been made of the concentration of hydrogen ions and of chloride ions in protein systems. Bugarzsky and Liebermann (4) measured the electromotive force between hydrogen electrodes immersed in a cell containing 0.05 normal hydrochloric acid and in one containing the same concentration of acid with varying amounts of egg albumin. They showed that the protein bound a part of the acid and obtained similar results on the alkaline side but could detect no binding of sodium chloride. They also pointed out that the fraction of acid or alkali bound was lowered as the concentration increased.

Blasel and Matula (2) used the formula: $n = N - \frac{C_{H^+}}{\alpha}$; where n is the amount of acid bound; N, the original normality; C_{H^+}, the determined hydrogen ion concentration at equilibrium; and α, the dissociation constant from specific conductivity data. Hoffman and Gortner (17) pointed out that the α used must be determined by potentiometric methods because the former

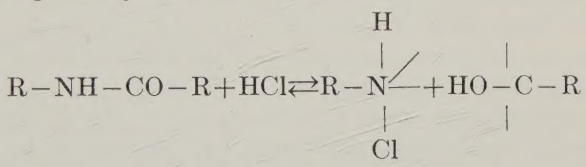
method gives lower values particularly on the alkaline side. Lloyd and Mays (28) consider that the quantity, $\sqrt{(H^+)\ (Cl^-)}$ gives a better value than the (H^+) in the equation of Blasel and Matula (2). They determined the value of (H^+) in the usual way, and calculated that the value of (Cl^-) by assuming that the gelatin chloride was completely ionized.

Chloride ion measurements have been made using a chlorine electrode. Working at concentrations below 0.05 normal, Bugarzsky and Liebermann (4) showed that almost as many chloride as hydrogen ions were bound. Manabe and Matula (29), Oryng and Pauli (34), and Hitchcock (16) have used the chlorine electrode in their studies. In general, it is found that at the lower concentrations of hydrochloric acid less chloride than hydrogen ions are bound and the difference, which they interpret as ionization of gelatin chloride, reaches a maximum at 0.02 normal hydrochloric acid. After that it drops, until at 0.05 normal ionization is completely suppressed and the two ions are bound in equivalent quantities.

Nature of the binding of acid and alkali. There are two schools of thought on the interpretation of the data in the literature. One group believes that the proteins bind acids and alkali stoichiometrically, that is, the primary valences of the basic groups neutralize the acid, yielding compounds analogous to ammonium salts. The other view explains the binding as an adsorption phenomenon following the empirical adsorption isotherm of Freundlich:

$$x = aC^{1/n}$$

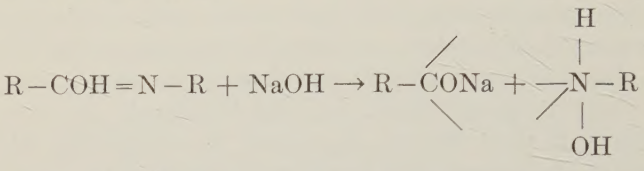
Evidence for chemical binding is based on the correlation between the amounts of acid bound and the basic groupings of the protein molecule, such as the ϵ-amino group of lysine, the guanidine nucleus of arginine, and possibly the imino group of the imidazole ring in histidine. Robertson (47, p. 208) believes that the peptide linkage functions in both acid and base binding. In the former case probably the keto form acts thus:



giving rise to two bulky protein ions. His postulate is based on the fact that, when one equivalent of acid is added and the solution electrolyzed, protein ions are found in both the anode and the cathode compartments. He later showed (48) that casein and gelatin when hydrolyzed by enzymes require less acid to bring the system to a pH of 2.0 than do the unhydrolyzed proteins. Therefore he argues that the basic groups do not bind the acid. He has forgotten the fact that hydrolysis also liberates acid groupings which will alter the buffer value of the system. His theory does not account for the fact that at low concentrations of acid added, the anions and cations are not bound in equivalent quantities.

In the chemical theory of alkali binding the quantity bound is correlated with the functional acidic groups present, according to the equation: dicarboxylic acid + OH group of tyrosine − amide nitrogen = base-binding equivalents.

Robertson believes that, as in the case with acids, the peptide linkage functions here but in the enolic form:



It is to be noted that none of the data supporting the theory of chemical binding extend beyond a pH 2.5 or 3.0 on the acid side and a pH of 11.0 in the basic range. Excellent summaries of the data leading to these conclusions are given by Hoffman and Gortner (17), and Cohn (5).

Workers who have extended the measurements beyond the field of the range of chemical binding are at a loss to account for the amounts of acid or alkali taken up by the proteins on the basis of primary valences. If the equivalents bound per gram of protein be plotted against either the initial concentrations of electrolyte or the equilibrium concentrations, curves very similar to adsorption isotherms result. Or better still, if the logarithms of the coordinates are used, straight lines result. Furthermore, these lines are very similar in spite of great differences in the chemical composition of the proteins. Hoffman and Gortner (17) also found a negative temperature coefficient between isotherms for 15°, 25°, and 35°, a fact which argues for adsorption in the ranges of hydrogen ion concentrations beyond pH = 2.5 and 10.5.

There are scattered references to the effect of other electrolytes on the acid- and base-binding capacities. Manabe and Matula (29) report that neutral salts caused a 2 to 3 per cent increase in the hydrogen ions bound by albumin and gelatin; also Blasel and Matula (2) found that salts increased the binding on both sides of neutrality. Oryng and Pauli (34) reported similar changes, and made the observation that both KCl and H_2SO_4 "considerably increased the chloride ions bound by serum in hydrochloric acid".

Csapo (6) repeated the work and found that sodium chloride, sodium iodide, and sodium nitrate increased by ten per cent the binding capacity of gelatin for hydrochloric, nitric, and sulfuric acids, while sodium sulfate depressed the binding 50 per cent. The series, K < Na < Ba < Ca, is reported for the cations. The author concludes that the effects are not due to common ion effects. The results produced suggest the typical Hofmeister series of ions affecting the imbibition of water by gelatin.

The binding of acid and alkali by globulins. Edestin, serum globulin and the Bence-Jones protein are the only globulins which have been studied. Osborne (36) pointed out the basic characteristic of edestin from which he was able to prepare two hydrochlorides. The one was soluble in water, the other insoluble; when titrated with standard alkali, using phenolphthalein

as an indicator, the former was found to require twice as much base as the second. He therefore considered the one edestin dihydrochloride, and the other the monohydrochloride.

Hardy (10) measured the quantity of various acids needed to dissolve one gram of serum globulin. He found that an average of 18×10^{-5} equivalents of the strong monobasic acids were required, twice that amount of sulfuric, tartaric, or oxalic acids were needed, and three times as much citric or phosphoric acid. From this he concluded that at saturation serum globulin combined in molecular rather than equivalent proportions. He did not measure the hydrogen ion concentration at equilibrium, but in the light of later work by Sörensen and others, probably all the acids ionized as monobasic compounds and he had reached the same degree of acidity in each case. Similarly, he found that mono-acid bases were bound by the protein at "saturation" to the extent of 10×10^{-5} gram equivalents, and the di-acid bases approximately twice as much.

Robertson (43, 44) repeated the work, using potentiometric methods to determine the hydrogen or hydroxyl ion concentrations of the equilibrium mixtures, and found that the acid equivalent increased with increasing acidity of the solution but that this was not so regular as on the alkaline side.

Hitchcock (13) showed that 0.45 grams edestin bound 5.6 cubic centimeters of 0.01 normal hydrochloric acid at a pH = 2.1 and an equivalent quantity of H_2SO_4, while oxalic and phosphoric acids were bound in the ratio of 1.9 and 3.2, respectively. In a later paper (15) he calculated the binding capacity of edestin for hydrochloric acid in a different way. His curve of hydrogen ions bound is plotted as the "total globulin chloride" and the curve showing the excess of free chloride ions over hydrogen ions is labeled "ionized globulin chloride". No measurements were made beyond an equilibrium pH = 1.1. He found the first curve to be nearly flat beyond a pH = 1.4 at which point the ionized salt had reached a maximum. From then on, the ionization dropped off very sharply.

Similar curves were drawn from the data on serum globulin but the conclusions arrived at were based on very scattered readings beyond a pH of 2.2 (p. 391). An earlier paper (14) reported the behavior of ox serum globulin with two alkalis, sodium hydroxide and barium hydroxide. A titration curve is given showing that the globulin binds equivalent quantities of either base, which he interprets as evidence of stoichiometrical binding. However, beyond a pH of 10 the data appear to us very much like those of a typical adsorption isotherm. Manabe and Matula (29) had earlier reported data of a very similar nature.

The isolectric point of edestin is reported at 1.3×10^{-7} N from a study of maximum precipitation in a phosphate buffer mixture (49). Later Michaelis and Mendelssohn (32) found the value of 2.5×10^{-6} N with acetate buffers. Hitchcock (14) also noted that the point of maximum precipitation varied with the buffer used.

The binding of acid and alkali by deaminized proteins. Blasel and Matula (2) showed that deaminized gelatin bound hydrogen ions to the extent of one half of that of the untreated gelatin. Hitchcock (16) repeated the work and showed that the acid equivalent of gelatin dropped from 8.9 to 4.0 $\times$ 10^{-5} when the protein was deaminized with nitrous acid. At the same time the protein lost 4.0 $\times$ 10^{-5} equivalents of amino nitrogen. Pauli and Hirschfeld (37) compared gelatin with its deaminized product, as did Blasel and Matula (2) who found that fewer hydrogen ions were bound by the treated product. On the alkaline side they found at the lower concentrations almost complete binding of the hydroxyl ion by all their proteins, with a constant increase of binding with increased addition of base.

The isolation and analysis of globulins. The globulins are defined as those naturally occurring proteins insoluble in water but soluble in neutral salt solutions. They are in general precipitated on dilution, on removal of the salts present by dialysis, and on complete saturation with magnesium sulfate, or by half saturation with ammonium sulfate. Like the albumins, the globulins are irreversibly heat-coagulable. Some well known members of animal origin are fibrinogen, serumglobulin, ovoglobulin and lactoglobulin. Serum globulin is often separated into two fractions; a smaller quantity, the euglobulin, precipitates out on diluting or dialyzing serum; but the major portion, pseudoglobulin, only precipitates when to the diluted serum is added an equal volume of saturated ammonium sulfate solution. No differences in the amino acid content on hydrolysis of the two fractions can be detected, but the euglobulin generally contains more phosphorus. In plants the globulins constitute a large part of the proteins of the seeds. Excelsin from the brazil nut, edestin from hemp seed, vicilin of the pea, arachin from the peanut, and glycinin from the soy bean are typical examples. Globulins as a group contain no amino acids in unusually large quantities with the exception of arginine which may constitute twenty per cent of the total nitrogen. They usually run fairly high in the dicarboxylic acids, largely glutamic acid, and the amide nitrogen is higher than the average of the proteins in general, but less than that of the prolamines.

Edestin was first prepared by Ritthausen (41) from hempseed, *Cannabis sativa*, using an alkaline extraction. Osborne (35) pointed out that the protein may be altered in the process and preferred to extract the fat-free meal with ten per cent sodium chloride. He removed the salt by dialysis and recrystallized the precipitate from warm 10 per cent sodium chloride in the ice box. This gives a fair yield of crystalline edestin and has become sufficiently standardized to be used in students' laboratory manuals. Reeves (39) used 0.5 normal sodium benzoate for the first extraction; for Schryver (52) had shown that at that concentration the surface tension of aqueous sodium benzoate was the lowest and the solution had the maximum peptizing action on edestin.

Mathews (30, p. 129) lists the following amino acids isolated from a hydrochloric acid hydrolysate, representing a 82.38 per cent recovery:

glycine	3.80	serine	0.53
alanine	3.60	cystine	1.00
valine	6.20	tyrosine	2.13
leucine	14.50	arginine	14.17
proline	4.10	histidine	2.19
phenylalanine	3.09	lysine	1.65
aspartic acid	4.50	ammonia	2.28
glutamic acid	18.84	tryptophane	+

Van Slyke (58) determined the nitrogen distribution which is compared with our analysis (Table I). More recently Schryver and his co-workers (53) have isolated from edestin β-hydroxylysine to the extent of 3.28 per cent of the total nitrogen. This should precipitate in the bases and would be calculated as lysine. Sakaguchi (50) believes that the guanidine nucleus of arginine is free in edestin since, by his colorimetric method, he obtained the same values in the hydrolysate as in the native protein. Hunter (19), on the other hand, finds that only one-half of the guanidine nucleus is attacked by arginase and so concludes that only that fraction is uncombined in edestin.

Ritthausen (40) first extracted a globulin from unroasted peanuts, *Arachis hypogaea*. Johns and Jones (20) repeated his work and separated the globulin into two fractions, arachin and conarachin, by fractional precipitation of the 10 per cent sodium chloride extract with ammonium sulfate. Arachin occurs in the larger quantity. In a later paper (21) they report the nitrogen distribution by the Van Slyke analysis.

The globulin of the cantaloupe seed, *Cucumis melo*, was first isolated by Jones and Gersdorff (22). They found it very similar to the squash seed globulin isolated according to the method of Osborne (35), as to nitrogen distribution, and identical immunologically.

The preparation and analysis of deaminized proteins. Skraup and Levites were the first to make any extensive studies on the reaction between nitrous acid and proteins. Levites (25) obtained an olive-green product by making a paste of sodium nitrite and the protein and then adding dilute acetic acid and warming. Skraup and Hoernes (54) preferred to dissolve or suspend the protein in dilute acetic acid, add sodium nitrite, and warm. They report an average yield of 70 per cent on a number of preparations. In this way they prepared deaminized casein; the same method was used by Traxl (57) to obtain deaminized edestin. Gelatin, albumin, and serum globulin were also deaminized in Skraup's laboratory. Levites (25, 26) deaminized egg albumin, gelatin, casein, gliadin, and vitellin.

Dunn and Lewis (7) made a study of the factors which influence the yield, and modified Skraup's method mainly in that by allowing the mixture to stand at room temperature for 18 hours they were able to obtain better yields and a more uniform product. They found that the insoluble product which settled out soon after adding the sodium nitrite was not completely deaminized but that 18 hours at a low temperature would allow the reaction to go to completion. Hitchcock (16) observed that only the free amino groups were

removed when the nitrous acid treatment was carried out at room temperature but that 100 per cent more nitrogen was lost when the reacting mixture was warmed. The preparations of Dunn and Lewis were yellow when freshly precipitated but turned brown on exposure to air. The material gave no free amino nitrogen in the Van Slyke apparatus. The biuret test was weaker than in the original casein, but the Hopkins-Cole test was not affected. These workers also verified Levites' (25) observation that Millon's test was positive altho somewhat weaker than in the untreated protein. Since Dunn and Lewis could isolate somewhat less tyrosine from the hydrolysate of the deaminized product and since quantitative measurements showed that approximately 50 per cent of the material responsible for the phenolic test remained, they concluded that tyrosine was not completely destroyed or altered by nitrous acid. Levites (26) had reported that the same quantity of all the mono-amino acids could be isolated from a deaminized protein as from the untreated protein, with the one exception of tyrosine which he was unable to isolate from any hydrolysate of deaminized proteins.

From a nitrogen distribution by Van Slyke's method Dunn and Lewis showed that practically all the lysine, half of the histidine, but none of the arginine is lost in the nitrous acid treatment. The filtrate from the bases gained proportionately in amino nitrogen. Their work confirms the theory that the ϵ-group of lysine is free in casein, and that this amino acid is converted by nitrous acid to the corresponding ϵ-hydroxy compound. However, this latter compound has never been isolated from the filtrate. Dunn and Lewis believe that the difference in histidine values is accidental since this amino acid is calculated by differences. Lampl (24) showed that arginine was unaltered, histidine only partially altered, and lysine completely changed, in deaminized serum globulin.

That the method of Dunn and Lewis does not radically alter the protein structure is indicated by the fact that enzymes hydrolyze deaminized casein in vitro, altho at a slower rate than casein (8). Similar results were obtained by Nakashima (33).

Experimental

The preparation and analysis of the proteins used. Edestin, arachin, and the cantaloupe seed globulin were selected as three proteins typical of their class and capable of being prepared in sufficient quantities for study. The two first mentioned were deaminized together with three of the proteins studied by Hoffman and Gortner (17). Durumin is a typical prolamine; casein and fibrin have been widely studied both in this laboratory and elsewhere. Of the eight proteins studied, deaminized durumin and deaminized fibrin have never been mentioned in the literature, none of the deaminized proteins except casein has ever been analyzed by the Van Slyke method, and data on the acid- and base-binding capacity of edestin only could be found.

The proteins were prepared according to the procedures given below. Ash, moisture, total nitrogen, and amino-nitrogen by the Van Slyke apparatus were determined in the usual way. In the Kjeldahl digestion, mercuric oxide was used as the catalyst and the mercury precipitated as its sulfide before

distillation. In a few cases the distillate was found to be sufficiently milky to make the end-point with sodium alizarin sulfonate difficult to determine. The distillate was then returned to Kjeldahl flask, made alkaline, and the ammonia redistilled into fresh standard acid.

For the nitrogen distribution 3 grams of protein were hydrolyzed 24 hours with constant-boiling hydrochloric acid. Van Slyke's (58) method was followed with the modifications used by Gortner and Sandstrom (9) except that no corrections were made for the solubility of the phosphotungstates of the bases. During the analysis of arachin the phosphotungstic acid was not completely removed in the first precipitation and the barium phosphotungstate was whiter than usual. On adding more barium chloride a second precipitate, much smaller than the first and of a dirty gray color, resulted. This observation may be of some interest in connection with the nature of the "phosphotungstic acid humin nitrogen", concerning which very little is known at the present time.

The preparation and analysis of arachin. Arachin was prepared from fat-free unroasted peanut meal (*Arachis hypogaea*) according to the method of Johns and Jones (20) with slight modifications. The meal was extracted several times with redistilled gasoline to remove the oil. Five hundred grams of the meal was stirred into three liters of 10 per cent sodium chloride. After five hours the liquid was squeezed thru two thicknesses of cheese-cloth. A second extraction yielded very little protein. The suspended starch and the small shreds of fibrous material were removed by passing the liquid through a supercentrifuge. The resulting opalescent solution was diluted with three volumes of distilled water and saturated with carbon dioxide. It was found desirable to add a little diphenyl ether as a foam inhibitor. The precipitate, which formed readily, redissolved in fresh 10 per cent sodium chloride with the exception of a small residue which was discarded. Johns and Jones found that the arachin fraction was completely precipitated at 0.2 saturation with ammonium sulfate. We observed a marked precipitation at that point but the mother liquors developed a turbidity which did not clear up after two days standing. Additional ammonium sulfate to make the total 0.25 saturated was added and the suspended arachin soon settled out, yielding a compact precipitate. This was washed with a solution of salts of the same concentration as the mother liquors. The precipitate was again dissolved in the least volume of 10 per cent sodium chloride and dialyzed until free from chlorides. The preparation was washed four times with ethyl alcohol of increasing strength, twice with ether, and dried at a low temperature. The preparation was then reduced to a fine powder in the ball mill. A yield of 28 per cent of the fat-free meal resulted.

	Found	Johns and Jones (20)
Moisture	6.68	
Ash	0.55	
Total nitrogen	16.91	
Total nitrogen on the ash- and moisture-free basis	18.19	18.29
Amino nitrogen	2.65	

The nitrogen distribution by Van Slyke's method is given in Table I where it is compared with that of Johns and Jones.

The isolation and analysis of edestin. Edestin was prepared from ground hempseed (*Cannabis sativa L.*) which had previously been extracted with gasoline. The first extraction was made with half normal sodium benzoate according to the method of Reeves (39), clarified in the super centrifuge and the protein precipitated by pouring the filtrate into twenty volumes of distilled water. This yields a precipitate which is easily washed by decantation, and does not appear to go over to edesten as rapidly as does edestin in sodium chloride solution. For purification the precipitate was dispersed in 10 per cent sodium chloride according to the method of Osborne (35), but not all of the precipitate could be redissolved in warm 10 per cent sodium chloride. The insoluble residue, which contained some starch, was discarded. The salt was partially removed by dialysis against running water; the last was removed by stirring up the precipitate with water and centrifuging. The protein was finally washed with alcohol and ether and dried as reported under arachin. The yield was 13 per cent of the fat-free meal.

A nitrogen distribution analysis was made on the hydrolysate of a 3 gram sample. This is reported in Table I where the results are compared with those of Van Slyke (59). The following analyses were also made:

	Found	Osborne (35)
Moisture	5.01	
Ash	1.18	
Total nitrogen	17.35	
Total nitrogen on ash- and moisture-free basis	18.50	18.70
Amino nitrogen	2.00	

The isolation and analysis of the globulin of the cantaloupe seed. The method of Jones and Gersdorff (22) was followed in isolating the globulin from cantaloupe seed* (*Cucumis melo*). The seeds were ground and most of the hulls removed by sifting. The oil was extracted with ether. Two hundred grams of meal was extracted with 2 liters of 2 per cent sodium chloride warmed to 60° C. The protein is easily and rapidly extracted, and crystallizes out on cooling the liquid. The globulin was purified by a second dispersion and precipitation. The yield was 48 per cent of the fat-free meal. Table I shows the nitrogen distribution on a 3 gram sample. The following analyses were also made:

	Found	Jones and Gersdorff (22)
Moisture	6.03	
Ash	1.25	
Total nitrogen	17.02	
Total nitrogen to the ash- and moisture-free basis	18.36	18.42
Amino nitrogen	1.78	

* We are indebted to Mr. John F. Trost of the Purdue Experiment Station who very kindly supplied us with the cantaloupe seed.

TABLE I

The Nitrogen Distribution of the Proteins used in This Study as determined by the Van Slyke Analysis and expressed in Per Cent of the Total Nitrogen

	Arachin		Deaminized Arachin	Edestin		Deaminized Edestin	Cantaloupe Seed Globulin		Durumin	Deaminized Durumin	Casein	Deaminized Casein		Fibrin	Deaminized Fibrin
	Johns and Jones (21)			Van Slyke (58)			Jones and Gersdorff (22)		Hoffman and Gortner (17)		Hoffman and Gortner (17)	Dunn and Lewis (8)		Hoffman and Gortner (17)	
Ammonia N	11.81	11.61	12.74	9.99	10.46	11.29	7.54	8.02	25.34	25.24	10.20	11.09	10.84	6.93	8.47
Humin N	0.57			1.98			0.70					2.85			
Acid insoluble		0.49	0.90		1.18	1.07		0.64	0.38	0.70	0.34		1.63	1.44	1.98
Acid soluble		0.28	0.14		0.68	0.50		0.22	0.46	0.54	1.17		1.07	1.48	1.64
Basic N															
Arginine	23.77	22.81	20.50	27.05	26.20	24.83	28.41	27.04	6.44	4.68	9.20	7.09	8.93	14.16	12.32
Histidine	2.78	1.64	1.36	5.75	4.14	3.90	6.20	4.10	3.88	3.00	6.26	3.89	5.41	4.30	3.41
Cystine	0.74	0.16	0.20	1.49	1.08	1.21	0.78	0.80	1.33	0.68	1.05	0.26	0.60	0.89	0.20
Lysine	5.22	5.00	0.10	3.86	3.60	0.30	3.41	3.28	1.88	0.16	8.49	0.67	0.64	14.06	0.42
Phosphotungstic acid N	0.43	0.29	0.39	—	0.40	0.50	0.08	0.21	—	0.40	—	—	0.39	—	0.53
Filtrate N															
Amino	53.30	54.30	59.12	47.55	48.73	52.26	50.45	53.08	53.98	57.16	54.12	66.50	60.12	55.86	66.17
Non-amino	1.65	3.18	3.83	1.70	2.58	4.38	2.14	2.63	6.53	6.96	8.76	5.85	9.84	0.23	3.68
Total	100.27	99.76	99.28	99.37	99.05	100.24	99.71	100.02	100.22	99.52	99.59	98.20	99.47	99.35	98.82

The preparation and analysis of the deaminized proteins. The deaminized proteins were all prepared in a uniform manner following the method of Dunn and Lewis (8). Fifty grams of the protein was suspended in 1 liter of distilled water. To this was added 70 c.c. glacial acetic acid drop by drop with continued stirring. Generally the proteins were completely peptized in 2 hours. When peptization was complete, 250 c.c. of a solution containing 20 grams of sodium nitrite was added slowly. A brownish-yellow precipitate soon formed, part of which settled out; the rest was caught by the escaping nitrogen gas and carried to the top. After standing for about 24 hours in the ice box the reaction mixture was allowed to warm to room temperature and was filtered on a Büchner funnel using hardened filter paper. Hot water was used to wash out the excess acid. Then alcohol was added. This appeared to liberate a small additional quantity of a gas, probably nitrogen. The alcohol extracted enough of a colored substance to color the filtrate a turbid brown. The protein was washed several times with alcohol, followed by 2 or 3 washings with ether and the product dried at a low temperature.

The material took on a cinnamon brown color on drying. The yields were uniformly good, giving over an 80 per cent recovery. Like the globulins, the products were ground in a ball mill until they were reduced to a fine powder. On drying the deaminized fibrin, a hard, vitreous mass resulted which required several hours treatment in the ball mill and resulted in an unusually high ash. A conductivity measurement was made on this product but its suspension gave practically the same value as the water used, indicating that the ash was porcelain dust from the ball mill.

The nitrogen distribution of the products is given in Table I, and for purposes of comparison, either the values in the literature or those of the untreated protein are included. Other analyses of the products are given in Table II.

TABLE II

The analyses of the deaminized proteins used in this study

	Ash	Moisture	Total Nitrogen	Total nitrogen on moisture- and ash-free basis
Deaminized arachin	0.48	6.10	16.39	17.55
Deaminized edestin	2.39	7.41	16.00	17.75
Deaminized casein	0.52	5.23	14.23	15.09
Deaminized fibrin	4.46	4.92	13.75	15.17
Deaminized durumin	0.28	5.67	15.98	16.99

Methods used in measuring the binding. The method used in determining the amount of acid bound is that used by Hoffman and Gortner (17). Exactly 0.1 gram of the protein was used in each case with the acid or alkali of the desired normality added. It was found desirable to make a paste of the protein with part of the solution and then transfer this to a 10 c.c. volumetric flask,

using the last of the solution as wash water. This procedure insured thoroughly wetting the finely divided powder. Half an hour was allowed for equilibrium to be reached and the measurements were made at 30°C. ± 0.5.

The Bailey electrode was found very convenient; equilibrium was reached in two or three minutes with shaking. A Leeds and Northrup potentiometer, Type K, was used with a Leeds and Northrup Type R high-sensitivity galvanometer. The potential was measured with the electrode in the series H_2, H^+ || KCl (N) Hg and Hg_2Cl_2. The millivolt readings were converted into hydrogen ion concentration and pH values from the tables of Schmidt and Hoagland (51).

The calculations of the equivalents of acid or alkali bound were made by the equation: $n = N - N'$, where n is the normality of acid bound by 0.1 gm. protein and contained in 10 c.c. solution, N is the original normality, and N′ the normality after equilibrium. N′ was read off a curve made by plotting the $C_H{}^+$ of varying normalities of HCl in water from the data of Tables III and IV. Experimental data are shown in Tables V to XX. As explained by Hoffman and Gortner (17) no temperature corrections need be introduced. From n, the equivalent bound per gram is obtained by dividing by 10 and multiplying by a correction factor to take care of the ash and moisture content of the protein.

It is to be noted that one assumption is made in calculating the binding capacity from potentiometric data. We have calculated the hydrogen ion concentration of a solution of hydrochloric acid or of sodium hydroxide in the presence of a protein on the assumption that the protein does not interfere and that a given E.M.F. indicates a given hydrogen ion concentration from which acid normality can be calculated. It is recognized that neutral salts affect the hydrogen ion concentration of dilute hydrochloric acid as shown by Harned (11, 12) and Brönsted and LaMer (3). However, we know of no measurements of the effects of electrolytes as weak as the proteins.

At this point it should be remarked that we have used the older terminology of "hydrogen ion concentration" instead of "hydrogen ion activity". The real relationship between the two is given by the expression:

Hydrogen ion activity = $\gamma \times$ hydrogen ion concentration, where γ is the activity coefficient of Lewis, Debye and others. In other words, we recognize that the potentials measured give us the intensity factor and not the quantity factor. Our usage may be justified on three grounds: first, in citing the older workers it is convenient to use their terminology; and second, at present the values for λ have not been accurately determined for values above 0.1 normal concentrations, whereas we extended our studies beyond that point. Also a recent paper by Beutner and Menitoff (1) showed that "electromotive forces * * * arise whenever the salt solutions are bordering on a salt-free layer containing a colloid" but that at present it is impossible to calculate the correction to be applied.

The experimental data are shown in Tables III-XX.

TABLE III

The potentiometric determination of the hydrogen ion concentration of varying normalities of HCl at 30°C.

N	E.M.F.	pH	C_{H^+}
0.002	435.9	2.723	0.00260
0.004	421.7	2.345	0.00453
0.008	402.7	2.024	0.00945
0.016	388.5	1.783	0.01650
0.024	379.0	1.623	0.0238
0.032	374.0	1.521	0.0301
0.040	368.7	1.449	0.0357
0.060	357.1	1.253	0.0559
0.080	348.0	1.099	0.0797
0.120	337.8	0.952	0.1184
0.160	331.1	0.813	0.1529
0.240	319.9	0.624	0.2379
0.360	309.6	0.450	0.3562

TABLE IV

The potentiometric determination of the hydroxyl ion concentration of varying concentrations of sodium hydroxide at 30°C.

N	E.M.F.	pH	C_{OH^-}
0.004	960.5	11.454	0.00288
0.010	981.4	11.807	0.00646
0.020	1005.4	12.213	0.01648
0.030	1013.1	12.343	0.0222
0.040	1018.0	12.426	0.0270
0.060	1028.5	12.604	0.0406
0.080	1036.6	12.740	0.0556
0.120	1044.9	12.880	0.0770
0.160	1052.9	13.015	0.1044
0.200	1057.9	13.100	0.1275
0.250	1061.1	13.155	0.1450
0.300	1064.3	13.208	0.1635
0.400	1071.7	13.334	0.2179
0.500	1076.0	13.406	0.2580

Table V

The potentiometric titration of arachin (1 per cent) with varying normalities of HCl at 30° C.

N	E.M.F.	pH	C_H^+	N′	n
———	478.0	3.296	0.506×10^{-3}	0.00048	———
0.002	467.0	3.111	0.775×10^{-3}	0.00065	0.00135
0.004	451.0	2.840	0.145×10^{-2}	0.0012	0.0028
0.008	429.5	2.477	0.334×10^{-2}	0.0029	0.0051
0.016	408.8	2.125	0.0075	0.007	0.009
0.024	397.0	1.927	0.0118	0.012	0.012
0.032	396.0	1.893	0.0128	0.013	0.019
0.071	376.0	1.572	0.0268	0.028	0.043
0.120	349.1	1.118	0.0763	0.080	0.040
0.180	337.0	0.913	0.1220	0.128	0.052
0.240	329.4	0.785	0.1644	0.170	0.070
0.300	321.3	0.647	0.2255	0.229	0.071

Table VI

The potentiometric titration of the globulin of the cantaloupe seed (1 per cent) with varying normalities of HCl at 30° C.

N	E.M.F.	pH	C_H^+	N′	n
———	558.5	4.648	0.22×10^{-4}	0.00002	———
0.002	515.5	3.931	0.118×10^{-3}	0.0001	0.0019
0.004	485.8	3.424	0.373×10^{-3}	0.0003	0.0037
0.008	446.0	2.756	0.176×10^{-2}	0.0016	0.0064
0.016	411.6	2.174	0.0067	0.006	0.010
0.024	406.5	2.087	0.817×10^{-2}	0.008	0.016
0.032	390.1	1.811	0.155×10^{-1}	0.016	0.016
0.080	358.5	1.277	0.053	0.056	0.024
0.120	347.8	1.096	0.0803	0.090	0.030
0.160	337.7	0.925	0.1188	0.124	0.036
0.240	327.8	0.758	0.1748	0.181	0.059
0.300	321.7	0.654	0.222	0.227	0.073

TABLE VII

The potentiometric titration of edestin (1 per cent) with varying normalities of HCl at 30°C.

N	E.M.F.	pH	C_H^+	N′	n
	659.7	6.346	0.43×10^{-6}		
0.002	561.0	4.700	0.00002	0.000018	0.00199
0.004	512.6	3.883	0.00013	0.00011	0.00389
0.008	484.7	3.410	0.0039	0.00032	0.00768
0.016	424.8	2.400	0.0040	0.0045	0.0115
0.024	403.5	2.038	0.0092	0.0090	0.015
0.032	386.4	1.736	0.0179	0.018	0.014
0.040	377.5	1.598	0.0253	0.026	0.014
0.080	356.2	1.137	0.058	0.061	0.019
0.120	344.3	1.036	0.092	0.097	0.023
0.160	336.3	0.901	0.126	0.131	0.029
0.240	324.2	0.700	0.201	0.206	0.034
0.300	317.8	0.589	0.258	0.263	0.037

TABLE VIII

The potentiometric titration of deaminized arachin (1 per cent) with varying normalities of HCl at 30°C.

N	E.M.F.	pH	C_H^+	N′	n
	468.0	3.128	0.746×10^{-3}	0.00068	
0.002	459.0	2.975	0.00106	0.0009	0.0011
0.004	440.0	2.654	0.00222	0.0018	0.0022
0.008	414.0	2.215	0.00611	0.0053	0.002
0.016	404.6	2.022	0.0095	0.009	0.007
0.024	392.0	1.843	0.01435	0.014	0.010
0.032	384.0	1.707	0.01965	0.021	0.011
0.071	364.0	1.367	0.04275	0.045	0.026
0.120	347.4	1.089	0.0816	0.086	0.034
0.180	335.9	0.894	0.1275	0.133	0.047
0.240	326.7	0.739	0.1825	0.189	0.051
0.300	319.9	0.624	0.2379	0.240	0.060

Table IX

The potentiometric titration of deaminized edestin (1 per cent) with varying normalities of HCl at 30° C.

N	E.M.F.	pH	C_H^+	N′	n
———	467.8	3.125	0.752×10^{-3}	———	———
0.002	452.1	2.859	0.00139	0.00105	0.001
0.004	434.6	2.563	0.00273	0.00204	0.00196
0.008	417.2	2.268	0.00539	0.005	0.003
0.016	399.5	1.969	0.0107	0.011	0.005
0.024	395.6	1.903	0.0125	0.014	0.010
0.040	376.8	1.523	0.0260	0.028	0.012
0.080	355.3	1.222	0.0597	0.063	0.017
0.120	343.8	1.028	0.0939	0.099	0.021
0.180	332.5	0.837	0.1458	0.151	0.029
0.240	325.2	0.713	0.1965	0.203	0.037
0.300	317.2	0.578	0.2640	0.273	0.027

Table X

The potentiometric titration of deaminized casein (1 per cent) with varying normalities of HCl at 30° C.

N	E.M.F.	pH	C_H^+	N′	n
———	530.7	4.189	0.658×10^{-4}	0.58×10^{-4}	———
0.002	449.9	2.821	0.00151	0.00115	0.00085
0.004	432.4	2.526	0.00298	0.0024	0.0016
0.008	412.8	2.195	0.00639	0.006	0.002
0.016	396.6	1.920	0.0120	0.011	0.005
0.024	388.5	1.784	0.0165	0.016	0.008
0.032	380.6	1.650	0.0224	0.023	0.009
0.040	375.3	1.560	0.0275	0.030	0.010
0.060	362.7	1.348	0.0449	0.048	0.012
0.080	354.6	1.210	0.0616	0.065	0.015
0.120	342.3	1.002	0.098	0.103	0.017
0.160	334.5	0.871	0.135	0.140	0.020
0.200	328.4	0.768	0.171	0.178	0.022
0.240	323.0	0.676	0.211	0.216	0.024
0.300	317.6	0.585	0.260	0.266	0.034
0.400	309.0	0.454	0.353	0.361	0.039

TABLE XI

The potentiometric titration of deaminized durumin (1 per cent) with varying concentrations of HCl at 30° C.

N	E.M.F.	pH	C_H^+	N′	n
———	512.6	3.881	$.1312 \times 10^{-3}$	———	———
0.002	452.1	2.857	$.1389 \times 10^{-2}$	0.0011	0.0009
0.004	432.7	2.531	$.2946 \times 10^{-2}$	0.0026	0.0014
0.008	412.0	2.181	.0066	0.0056	0.0024
0.016	397.2	1.930	.0117	0.0118	0.004
0.024	383.3	1.696	.0202	0.0203	0.004
0.032	378.0	1.606	.0248	0.026	0.006
0.040	374.2	1.545	.0287	0.030	0.010
0.080	354.3	1.180	.0649	0.068	0.012
0.120	339.9	0.978	.1046	0.106	0.014
0.180	330.3	0.787	.1587	0.166	0.014
0.240	322.1	0.661	.218	0.220	0.020
0.300	320.6	0.634	.250	0.252	0.048

TABLE XII

The potentiometric titration of deaminized fibrin (1 per cent) with varying normalities of HCl at 30° C.

N	E.M.F.	pH	C_H^+	N′	n
———	495.9	3.599	0.252×10^{-3}	———	———
0.002	458.2	2.962	0.1092×10^{-2}	0.00084	0.0012
0.004	443.5	2.713	0.1935×10^{-2}	0.00149	0.0025
0.008	416.8	2.212	0.5474×10^{-2}	0.00483	0.0032
0.016	395.7	1.905	0.01245	0.0130	0.003
0.024	386.0	1.741	0.0182	0.0194	0.005
0.032	376.9	1.582	0.0257	0.0276	0.005
0.040	369.5	1.445	0.0345	0.0360	0.004
0.080	351.9	1.165	0.0659	0.0695	0.011
0.120	343.4	1.022	0.0954	0.1000	0.020
0.180	332.5	0.837	0.1458	0.1515	0.029
0.240	325.2	0.713	0.1935	0.2000	0.040
0.300	318.5	0.601	0.2510	0.2620	0.038

Table XIII

The potentiometric titration of arachin (1 per cent) with varying normalities of NaOH at 30° C.

N	E.M.F.	pH	C_{OH^-}	N′	n
0.004	731.0	7.574	3.94×10^{-7}	5.2×10^{-7}	0.004
0.010	812.4	8.950	9.008×10^{-6}	0.000012	0.010
0.020	957.5	11.403	2.56×10^{-3}	0.005	0.015
0.030	982.9	11.832	6.85×10^{-3}	0.013	0.017
0.040	999.8	12.118	0.0133	0.020	0.020
0.060	1012.8	12.338	0.0220	0.035	0.025
0.080	1023.5	12.519	0.0335	0.049	0.031
0.120	1032.6	12.672	0.048	0.073	0.047
0.160	1040.2	12.801	0.064	0.098	0.062
0.200	1050.4	12.974	0.095	0.147	0.053
0.250	1054.6	13.044	0.112	0.179	0.072
0.300	1055.2	13.054	0.122	0.198	0.102
0.350	1065.4	13.227	0.173	0.296	0.104
0.400	1069.1	13.300	0.197	0.351	0.129

Table XIV

The potentiometric titration of the cantaloupe seed globulin (1 per cent) with varying normalities of sodium hydroxide at 30° C.

N	E.M.F.	pH	C_{OH^-}	N′	n
0.004	785.1	8.489	3.112×10^{-6}	———	0.004
0.010	890.5	10.271	1.885×10^{-4}	———	0.010
0.020	968.4	11.587	0.00391	0.008	0.012
0.030	989.5	11.944	0.00891	0.012	0.018
0.040	998.9	12.103	0.01285	0.0185	0.022
0.060	1010.6	12.300	0.0202	0.0295	0.031
0.080	1024.8	12.541	0.0351	0.051	0.029
0.120	1040.5	12.807	0.0648	0.089	0.031
0.160	1044.9	12.880	0.0770	0.120	0.040
0.200	1050.9	12.981	0.0975	0.153	0.047
0.250	1055.3	13.056	0.1154	0.184	0.066
0.300	1059.7	13.134	0.1368	0.226	0.074
0.350	1061.4	13.160	0.1462	0.245	0.105
0.500	1070.2	13.308	0.2056	0.372	0.128

TABLE XV

The potentiometric titration of edestin (1 per cent) with varying concentrations of sodium hydroxide at 30° C.

N	E.M.F.	pH	C_{OH^-}	N′	n
0.004	907.8	10.563	3.706×10^{-4}	0.0004	0.0036
0.010	954.1	11.348	2.244×10^{-3}	0.0034	0.0066
0.020	984.7	11.846	7.076×10^{-3}	0.011	0.009
0.030	995.4	12.078	1.123×10^{-2}	0.015	0.015
0.040	1007.7	12.252	1.809×10^{-2}	0.025	0.015
0.060	1017.6	12.419	0.0265	0.040	0.020
0.080	1020.5	12.469	0.0297	0.044	0.036
0.120	1037.7	12.759	0.0581	0.078	0.042
0.160	1043.4	12.855	0.0741	0.114	0.046
0.200	1049.8	12.964	0.0932	0.146	0.054
0.250	1054.9	13.049	0.1136	0.182	0.068
0.300	1056.8	13.082	0.1221	0.199	0.101
0.350	1062.5	13.179	0.1522	0.258	0.092
0.400	1063.1	13.188	0.1557	0.265	0.135

TABLE XVI

The potentiometric titration of deaminized arachin (1 per cent) with varying normalities of NaOH at 30° C.

N	E.M.F.	pH	C_{OH^-}	N′	n
0.004	736.0	7.658	4.6×10^{-7}	1×10^{-6}	0.004
0.010	886.0	10.204	1.624×10^{-4}	2.7×10^{-4}	0.0097
0.020	975.4	11.706	5.14×10^{-3}	0.0062	0.0138
0.030	998.9	12.102	0.01285	0.0185	0.0115
0.040	1005.3	12.211	0.0164	0.024	0.016
0.060	1011.0	12.307	0.0205	0.039	0.021
0.080	1026.4	12.568	0.0374	0.058	0.022
0.120	1032.2	12.682	0.0488	0.074	0.046
0.160	1041.8	12.828	0.0682	0.110	0.050
0.200	1049.2	12.953	0.0911	0.142	0.058
0.250	1054.3	13.039	0.1109	0.177	0.073
0.300	1059.2	13.122	0.1340	0.222	0.078
0.350	1063.0	13.186	0.1550	0.263	0.087
0.400	1067.3	13.259	0.1841	0.328	0.072

Table XVII

The potentiometric titration of deaminized edestin (1 per cent) with varying normalities of NaOH at 30°C.

N	E.M.F.	pH	C_{OH^-}	N′	n
0.004	710.0	7.219	1.68×10^{-7}	2×10^{-7}	0.004
0.010	878.7	10.071	1.19×10^{-4}	1.7×10^{-4}	0.010
0.020	971.0	11.631	4.32×10^{-3}	0.0048	0.0152
0.030	979.7	11.778	6.07×10^{-3}	0.0127	0.0173
0.040	998.7	12.101	0.01275	0.019	0.021
0.060	1012.8	12.337	0.0222	0.035	0.025
0.080	1024.7	12.539	0.0350	0.052	0.028
0.120	1033.8	12.693	0.0499	0.076	0.044
0.160	1043.3	12.853	0.0722	0.112	0.048
0.180	1047.5	12.925	0.0853	0.132	0.048
0.200	1048.9	12.949	0.0901	0.141	0.059
0.250	1055.9	13.053	0.1180	0.181	0.069
0.300	1058.2	13.088	0.1288	0.210	0.090
0.350	1061.5	13.161	0.1473	0.247	0.103
0.400	1064.2	13.206	0.1640	0.282	0.118

Table XVIII

The potentiometric titration of deaminized casein (1 per cent) with varying concentrations of NaOH at 30°C.

N	E.M.F.	pH	C_{OH^-}	N′	n
0.004	720.9	7.403	2.56×10^{-7}	2×10^{-7}	0.004
0.010	755.9	7.994	9.98×10^{-7}	7.4×10^{-7}	0.010
0.020	945.5	11.200	1.61×10^{-3}	0.002	0.018
0.030	979.9	11.781	6.12×10^{-3}	0.009	0.021
0.040	995.4	12.044	0.0112	0.016	0.024
0.060	1013.3	12.346	0.0225	0.033	0.027
0.080	1024.9	12.542	0.0353	0.052	0.028
0.120	1038.3	12.769	0.0594	0.090	0.030
0.160	1046.1	12.901	0.0807	0.125	0.035
0.180	1047.9	12.931	0.0867	0.134	0.046
0.200	1051.2	12.986	0.0980	0.153	0.047
0.250	1056.2	13.071	0.1194	0.193	0.057
0.300	1062.7	13.182	0.1534	0.259	0.041
0.350	1064.2	13.206	0.1628	0.279	0.071
0.400	1066.8	13.251	0.1809	0.320	0.080

TABLE XIX

The potentiometric titration of deaminized durumin (1 per cent) with varying concentrations of NaOH at 30°C.

N	E.M.F.	pH	C_{OH^-}	N′	n
0.004	907.8	10.563	3.706×10^{-4}	0.0004	0.0036
0.010	957.1	11.396	2.52×10^{-3}	0.0047	0.0053
0.020	992.5	11.995	0.0068	0.0120	0.008
0.030	1003.8	12.185	0.0155	0.022	0.008
0.040	1012.7	12.336	0.0218	0.031	0.009
0.060	1022.3	12.498	0.0320	0.050	0.010
0.080	1031.6	12.655	0.0458	0.068	0.012
0.120	1041.2	12.815	0.0672	0.104	0.016
0.160	1047.3	12.921	0.0846	0.132	0.028
0.200	1052.1	13.002	0.1001	0.158	0.042
0.250	1059.2	13.122	0.1341	0.220	0.030
0.300	1061.5	13.195	0.1468	0.247	0.053
0.350	1065.0	13.220	0.1680	0.291	0.059
0.400	1068.9	13.286	0.1950	0.337	0.063

TABLE XX

The potentiometric titration of deaminized fibrin (1 per cent) with varying normalities of NaOH at 30°C.

N	E.M.F.	pH	C_{OH^-}	N′	n
0.004	707.6	7.178	1.526×10^{-7}	2×10^{-7}	0.004
0.010	861.0	9.772	5.99×10^{-5}	8×10^{-5}	0.010
0.020	962.0	11.479	3.05×10^{-3}	0.0045	0.0155
0.030	983.9	11.849	7.12×10^{-3}	0.010	0.020
0.040	997.8	12.085	0.0123	0.015	0.025
0.060	1006.8	12.237	0.0174	0.024	0.036
0.080	1029.7	12.624	0.0425	0.060	0.020
0.120	1041.1	12.816	0.0664	0.101	0.019
0.160	1047.0	12.916	0.0836	0.128	0.032
0.200	1052.1	13.002	0.1015	0.155	0.045
0.250	1056.5	13.077	0.1208	0.196	0.052
0.300	1061.1	13.155	0.1445	0.241	0.059
0.350	1063.2	13.189	0.1560	0.266	0.084
0.400	1065.4	13.227	0.1709	0.297	0.103

IV. Discussion

The preparation and analysis of the proteins used. The three globulins were prepared and analyzed according to the method of Van Slyke (58). Our findings agree very well with those reported in the literature. It is to be noted that as a group of proteins they are uniformly high in arginine, and differ from the prolamines studied by Hoffman and Gortner (17) in that the globulins contain only 50 per cent of the amide nitrogen of the prolamines and 2 or 3 times as much lysine nitrogen.

The method of Dunn and Lewis (7) proved to be very satisfactory in preparing the deaminized proteins in good yields. A comparison of the nitrogen distribution in each of the deaminized proteins with that of the untreated proteins shows clearly that, with the possible exception of the cystine and the histidine fractions, only the lysine nitrogen is altered. This confirms the theory of Van Slyke and Birchard (59) that the ϵ-amino group in lysine is free in native proteins and loses its nitrogen when treated with an excess of nitrous acid. In keeping with their theory, the filtrate from the bases should gain one-half of the total nitrogen of lysine. If our values are corrected for the solubility of the bases, as is the usual procedure, we find that from 40 to 65 per cent of the total lysine nitrogen appears in the filtrate from the bases.

The amide nitrogen and the arginine values are not altered by the nitrous acid treatment, and the histidine fraction does not appear to be greatly changed by the process. Dunn and Lewis report a lower value in their deaminized casein which they ascribe to errors in analysis.

Although our deaminized proteins did not liberate any nitrogen in the Van Slyke apparatus, the analysis of the hydrolysates gave a calculated value of lysine nitrogen of from 0.1 to 0.6 per cent. These values are low in comparison with the total lysine nitrogen of the native proteins and are not greatly in excess of the errors found by Gortner and Sandstrom (9) in a study of the accuracy of the Van Slyke nitrogen distribution of a protein.

The values for cystine nitrogen of the deaminized proteins by the barium sulfate method are uniformly lower than those of the corresponding native proteins, and may indicate a partial conversion of cystine to cysteic acid with nitrous acid, since Hopkins and Dixon (18) and Kendall and Nord (23) have shown that cystine is very readily oxidized.

The binding of acid and alkali by the proteins. The values for the amount of acid or alkali bound were plotted as functions of the equilibrium concentrations. These were compared with the curves resulting when the logarithmic values were substituted. Since a preliminary inspection seemed to indicate that we were dealing with curves that looked very much like adsorption curves beyond the first two or three points plotted, it was thought desirable to calculate the values for the constants, a and b, in the equation:

$$\log n = \log a + b \log N'.$$

The "method of averages" as given by Lipka (27, p. 126) was used. This method consists in calculating the values for the constants a and b of this

straight line formula, such that the algebraic sum of the residuals is equal to zero. The data is divided into 2 groups and the pair of simultaneous equations constructed:

$$\begin{cases} \Sigma(\log n) = r \log a + b\,\Sigma\,(\log N') \\ \Sigma(\log n) = r \log a + b\,\Sigma\,(\log N') \end{cases}$$

where r is the number of observations in the group. From these equations log a and b are calculated. The values thus obtained from the data of Tables V-XX are given in Tables XXI and XXII.

TABLE XXI

The values of the constants, log a and b, in the equation: log n = log a + b log N′, for the binding of hydrochloric acid by the various proteins, as calculated from the data of Tables V-XII. (T. 30°C.)

	log a	b
Arachin	−0.46077	0.7581
Cantaloupe seed globulin	−0.3837	0.6087
Edestin	−1.4209	0.20656
Deaminized arachin	−0.62794	0.7914
Deaminized edestin	−0.5051	1.0197
Deaminized casein	−0.35409	1.2507
Deaminized fibrin	−1.5397	0.5058
Deaminized durumin	0.9440	0.8241

TABLE XXII

The values of the constants, log a and b, in the equation: log n = a + b log N′ for the binding of sodium, hydroxide by the various proteins, as calculated from the data of Tables XIII to XX. (T 30°C.)

	log a	b
Arachin	−0.8218	0.4522
Edestin	−0.6344	0.6477
Cantaloupe seed globulin	−0.7538	0.5617
Deaminized arachin	−1.0548	0.3376
Deaminized edestin	−0.2259	1.0394
Deaminized casein	−1.0122	0.3613
Deaminized fibrin	−0.8275	0.5268
Deaminized durumin	−1.2374	0.4122

The constant b of the logarithmic curve gives the slope of the line and is an index of the rate of change in the binding with varying acidity or alkalinity of the equilibrium system. On the acid side the values for the globulins vary from 0.2066 to 0.7578 and are lower than the values previously determined in this laboratory for the prolamines which gave values of from −0.6921 to −0.9613. It is to be noted that the values of Hoffman and Gortner (17) are negative due to the fact that they used the pH of the equilibrium concen-

trations as the one variable. The values for casein and fibrin with HCl are lower than those for the prolamines and fall within the range of the globulins.

On the alkaline side the values of b for the three globulins lie very close together (0.4522 to 0.6477) and are consistently lower than the corresponding values for the prolamines and fibrin and casein.

There is no regularity in the effects of deamination on the calculated slopes. A comparison of the constants of log a as between arachin or edestin and their deaminized products show that the values are lowered. Probably this may be explained on the basis of the lessened basicity of the deaminized products with less binding of acid in the lower concentrations.

A comparison was made between the globulins and their deaminized derivatives with regard to the amount of acid bound at the equilibrium pH of 2.5. The values for casein, fibrin, and durumin are taken from the data of Hoffman and Gortner (17). To make conditions more nearly comparable the mean values of their data for 22° and 35° were taken for comparison with our values which were obtained at 30°. This comparison is shown in Table XXIII. Column 2 indicates the gram equivalents of acid bound per gram of protein; column 3 gives the corresponding value for the deaminized product; column 4 is obtained by subtracting the values of column 3 from those of column 2, and represents the equivalents of acid-binding capacity lost by deamination. These values may be compared with the lysine nitrogen as per cent of the total nitrogen in the original protein (column 5). With the exception of edestin we find a striking parallelism between the lysine nitrogen content and the capacity of a protein to bind hydrochloric acid over that of the deaminized product.

TABLE XXIII

Showing the effect of deaminization on the acid-binding capacity of the proteins

Protein	Gram equivalents of acid bound per gram orig. protein	Gram equivalents of acid bound per gram deaminized protein	Gram equivalents of acid-binding lost by deaminization	Lysine N as per cent of total N in orig. protein
Edestin	0.00115	0.00020	0.00095	3.60
Fibrin	0.00096	0.00025	0.00071	14.06
Casein	0.00085	0.00016	0.00069	8.49
Arachin	0.00051	0.00022	0.00029	5.00
Durumin	0.00034	0.00014	0.00020	1.88
Cantaloupe seed globulin	0.00076	—	—	3.28

Possibly our lysine value for edestin is low, since Plimmer (38, p. 115) reports a lysine content of 1.7 per cent of the amino acid on actual isolation and Schryver (53) reports a value of 3.28 for the nitrogen percentage of β-hydroxylysine also obtained by isolation. These are undoubtedly minimum values. However, our value agrees well with that of Van Slyke. It

is evident from a comparison of the figures in columns 2 and 5 of Table XXIII that the ϵ-amino group of lysine is not alone responsible for acid-binding capacity. One would judge from Table XXIII that nitrous acid does more than remove the ϵ-amino group of lysine, since the acid-binding capacity of the deaminized proteins is very nearly uniform in spite of the great differences in acid-binding capacity of the native proteins.

Table XXIII, taken in conjunction with Table I, indicates that in this series of proteins, or at least in edestin, there is present some basic group other than the ϵ-amino group of lysine which binds acid. In the earlier study of the prolamines Hoffman and Gortner (17) found a correlation coefficient of $r = 0.992 \pm 0.003$ between the free amino nitrogen of the protein (the ϵ-group of lysine) and the gram equivalents of acid bound at pH 2.8. It is obvious that no such relationship applies to edestin. Neither does it appear that the arginine or histidine content of edestin can be responsible for its singularly high acid-binding capacity, for the histidine and lysine content is essentially the same in both the cantaloupe seed globulin and in edestin, and the arginine content is even higher in the cantaloupe seed globulin than in edestin. In spite of these relationships the acid-binding capacity of the cantaloupe seed globulin is much lower than is the case for edestin.

The available analyses fail to indicate any explanation for the high acid binding of edestin, although it will be noted that it must be due to some group which is destroyed by the treatment with nitrous acid.

Just the reverse effect of deamination is to be found on the alkaline side. Deamination shifts the isoelectric point of the protein to the acid side, and, with the exception of arachin, somewhat more alkali is bound by the deaminized proteins at a pH of 10.5 than is the case with the natural protein. Table XXIV shows this very clearly.

The gain of 200 per cent in the base-binding capacity of edestin and durumin, and of 50 per cent in the case of fibrin cannot be accounted for on the hypothesis that base binding by proteins is purely stoichiometrical and involves only combination of carboxyl groups with the base. In the case of a deaminized protein there are, presumably, no more free carboxyl groups than

TABLE XXIV

Showing the effect of deamination on the alkali-binding capacity of the proteins

Protein	Gram equivalents of alkali bound per gram orig. protein	Gram equivalents of alkali bound per gram deaminized protein	Change in gram equivalents of alkali binding due to deamination
Arachin	0.00112	0.00103	−0.00009
Edestin	0.00036	0.00111	+0.00075
Casein	0.00146	0.00163	+0.00017
Fibrin	0.00086	0.00118	+0.00032
Durumin	0.00012	0.00036	+0.00024
Cantaloupe seed globulin	0.00102	—	—

were present in the native protein and accordingly the base binding should not be greatly changed following deaminization. That it is changed for certain of the proteins is evident; however, there is as yet no satisfactory explanation for the fact that the base-binding capacities of edestin and fibrin are markedly increased by deaminization whereas those of arachin and casein are only slightly altered.

V. Summary and Conclusions

1. Edestin, arachin, and the globulin of the cantaloupe seed were prepared and analyzed for their nitrogen distribution by the method of Van Slyke.

2. Edestin, arachin, casein, fibrin, and durumin were deaminized and the resulting products analyzed by determining their nitrogen distribution.

3. The free amino group of lysine is attacked by the nitrous acid with the result that the lysine fraction is lost to the phosphotungstic acid fraction.

4. Probably histidine and cystine are slightly altered by the treatment with nitrous acid.

5. Varying concentrations of acid and alkali were added to the proteins and the potentiometric determinations of the hydrogen ion concentrations were made on the system at equilibrium.

6. From a preliminary inspection of the curves resulting when the quantity of acid or alkali bound was plotted as a function of equilibrium concentrations, it appeared that our proteins behaved very similarly to the prolamines previously studied in this laboratory.

7. With the exception of edestin, the quantity of acid bound by the proteins is roughly proportional to the lysine content of the proteins.

8. It is postulated that some basic group other than the ϵ-amino group of lysine must be responsible for the acid-binding capacity of edestin.

9. For all five proteins studied the acid-binding capacity is greatly decreased when the proteins are deaminized.

10. The quantities of sodium hydroxide bound at pH = 10.5 by the native proteins and their deaminized products were compared, and it was found that in four of the five cases more alkali was needed to bring the deaminized protein to that hydroxyl ion concentration than was required for the untreated protein.

11. From the data beyond the range of pH 2.5 and 10.5 the constants, log a and b, were calculated from the logarithmic values.

12. The constants for the globulins and the group of deaminized proteins differ amongst themselves but are very similar to the values obtained in this laboratory for the prolamines, except that the values for b, the slope of the line, for the globulins are uniformly lower than those for the prolamines.

13. As in the case of the prolamines this latter type of binding cannot be accounted for on the basis of primary valences, and since the empirically derived Freundlich adsorption isotherm can be made to fit the data, we conclude that in these regions we are dealing with typical adsorption phenomena.

VI. Literature Cited

(1) Beutner, R., and Menitoff, A.
1927. Influence of salt content of colloids upon their electromotive forces which may explain bioelectric currents. J. Biol. Chem., **72**, 759-763.

(2) Blasel, L., and Matula, J.
1914. Untersuchungen über physikalische Zustandsänderungen der Kolloide. XVI. Versuche am Desamidoglutin. Biochem. Z., **58**, 417-450, illus.

(3) Brönsted, J. N., and LaMer, V. K.
1924. The activity coefficients of ions in very dilute solutions. J. Am. Chem. Soc. **46**, 555-573, illus.

(4) Bugarzsky, S., and Liebermann, L.
1898. Über das Bindungsvermögen eiweissartiger Körper für Salzsäure, Natrium-hydroxyd und Kochsalz. Pflüger's Arch. für die gesamte Physiol., **72**, 51-74.

(5) Cohn, E. J.
1925. The physical chemistry of the proteins. Physiol. Rev., **5**, 349-437, illus.

(6) Csapo, J.
1923. Einfluss der Neutralsalze auf die Säurebindung der Gelatine. Biochem. Z., **159**, 53-57, illus.

(7) Dunn, M. S., and Lewis, H. B.
1921. The action of nitrous acid on casein. J. Biol. Chem., **491**, 327-341.

(8) Dunn, M. S., and Lewis, H. B.
1921. A comparative study of the hydrolysis of casein and deaminized casein by proteolytic enzymes. J. Biol. Chem., **49**, 342-350, illus.

(9) Gortner, R. A., and Sandstrom, W. M.
1925. Proline and trytophan as factors influencing the accuracy of Van Slyke's method for the determination of nitrogen distribution in proteins. J. Am. Chem. Soc., **47**, 1663-1671.

(10) Hardy, W. B.
1905. Colloidal solutions—The globulins. J. Physiol., **33**, 251-337, illus.

(11) Harned, H. S.
1920. The thermodynamic properties of the ions of some strong electrolytes and of the hydrogen ion in solutions of tenth molar hydrochloric acid containing uni-valent salts. J. Am. Chem. Soc., **42**, 1808-1832, illus.

(12) Harned, H. S.
1925. The activity coefficient of sodium hydroxide in sodium chloride solutions. J. Am. Chem. Soc., **47**, 684-689, illus.

(13) Hitchcock, D. I.
1922. The colloidal behavior of edestin. J. Gen. Physiol., **4**, 597.

(14) Hitchcock, D. I.
1922. The colloidal behavior of serum globulin. J. Gen. Physiol., **5**, 35-44, illus.

(15) Hitchcock, D. I.
1923. The ionization of protein chlorides. J. Gen. Physiol., **5**, 383-394, illus.

(16) Hitchcock, D. I.
1923. The combination of deaminized gelatin with hydrochloric acid. J. Gen. Physiol., **6**, 95-104, illus.

(17) Hoffman, W. F., and Gortner, R. A.
1925. Physico-chemical studies on proteins. I. The prolamines—Their chemical composition in relation to acid and alkali binding. *In* Holmes, H. N. (ed.): "Colloid Symposium Monograph," **2**, 209-368, Illus.

(18) Hopkins, F. G., and Dixon, M.
1922. On glutathione. II. A thermostable oxidation-reduction system. J. Biol. Chem., **54**, 527-563, illus.

(19) Hunter, A.
1925. Protein structure and proteolysis in some of their recent aspects. Trans. Roy. Soc. Canada, **19**, Sec. V, 1-23, illus.

(20) Johns, C. O., and Jones, D. B.
1916. The proteins of the peanut, *Arachis hypogaea*. I. The globulins arachin and conarachin. J. Biol. Chem., **28**, 77-87.

(21) Johns, C. O., and Jones, D. B.
1917. The proteins of the peanut, *Arachis hypogaea*. II. The distribution of the basic nitrogen in the globulins arachin and conarachin. J. Biol. Chem., **30**, 33-38.

(22) Jones, D. B., and Gersdorff, C. E. F.
1923. Proteins of the cantaloupe seed, *Cucumis melo*. J. Biol. Chem., **56**, 79-96, illus.

(23) Kendall, E. C., and Nord, F. F.
1926. Reversible oxidation-reduction systems of cysteine-cystine and reduced and oxidized glutathione. J. Biol. Chem., **69**, 295-337, illus.

(24) Lampl, H.
1907. Über Desamidoedestin. Monatsheft, **28**, 625-632.

(25) Levites, S. J.
1904. Über Desamidoalbumine. Hoppe-Seyler's Z. physiol. Chem., **43**, 202-206.

(26) Levites, S. J.
1909. Über Desamidoproteine. Biochem. Z., **20**, 224-230.

(27) Lipka, J.
1921. "Graphical and mechanical computation." Pt. II. Experimental data. 264 p., illus.

(28) Lloyd, D. J., and Mays, C.
1922. The titration curve of gelatine. Proc. Roy. Soc., **93B**, 69-85, illus.

(29) Manabe, K., and Matula, J.
1913. Untersuchungen über physikalische Zustandsänderungen der Kolloide. XV. Elektrochemische Untersuchungen am Säureeiweiss. Biochem. Z., **52**, 369-408, illus.

(30) Mathews, A. P.
1920. "Physiological chemistry," Ed. 3. 1154 p.

(31) Mellanby, J.
1905. Globulins. J. Physiol., **33**, 338-373, illus.

(32) Michaelis, L., and Mendelssohn, A.
1914. Die Wirkungsbedingungen des Pepsins. Biochem. Z., **65**, 1-15, illus.

(33) Nakashima, R.
1925. Über die Verdaulichkeit der Desaminoproteine durch proteolytische Fermente. J. Biochem. (Tokyo), **5**, 293-310.

(34) Oryng, T., and Pauli, W.
1915. Über Neutralsalzeiweissverbindungen. Biochem. Z., **70**, 368-397, illus.

(35) Osborne, T. B.
1892. Crystallised vegetable proteids. Am. Chem. J., **14**, 662-689.

(36) Osborne, T. B.
1902. The basic character of the protein molecule and the reaction of edestin with definite quantities of acids and alkalies. J. Am. Chem. Soc., **24**, 39-78.

(37) Pauli, W., and Hirschfeld, M.
1914. Die Proteinsalze verschiedener Säuren. Biochem. Z., **62**, 245-265, illus.

(38) Plimmer, R. H. A.
1917. "The chemical constitution of the proteins." Ed. 2. 174 p.

(39) Reeves, G.
1915. A new method for the preparation of the plant globulins. Biochem. J., 9, 508-510.

(40) Ritthausen, H.
1880. Über die Eiweisskörper verschiedener Ölsamen. Pflüger's Arch. für die gesamte Physiol., **21**, 81-104.

(41) Ritthausen, H.
1881. Krystallinische Eiweisskörper aus verschiedenen Ölsamen. J. prakt. Chem., **23**, 481-486.

(42) Robertson, T. B.
1906. On the conditions of equilibrium of an associating amphoteric electrolyte in the presence of any number of non-amphoteric electrolytes. J. Phys. Chem., **10**, 524-582.

(43) Robertson, T. B.
1907. On the dissociation of serum globulin at varying hydrogen ion concentrations. J. Phys. Chem., **11**, 437-459.

(44) Robertson, T. B.
1908. Note on the applicability of the laws of amphoteric electrolytes to serum globulin. J. Biol. Chem., **5**, 155-161.

(45) Robertson, T. B.
1910. The dissociation of potassium caseinate in solutions of varying alkalinity. J. Phys. Chem., **14**, 528-568.

(46) Robertson, T. B.
1911. The dissociation of the salts of ovo-mucoid in solutions of varying alkalinity and acidity. J. Phys. Chem., **14**, 709-718.

(47) Robertson, T. B.
1920. "The physical chemistry of the proteins." 483 p.

(48) Robertson, T. B.
1924. The influence of hydrolysis upon the capacity of proteins to bind acids and bases. Australian J. Exp. Biol. Med. Sci., **1**, 31-37, illus.

(49) Rona, P., and Michaelis, L.
1910. Über die Fällung der Globuline im isoelektrischen Punkt. Biochem. Z., **28**, 193-199.

(50) Sakaguchi, S.
1925. Über die Bindungsweise und quantitative Bestimmung des Arginins im Proteinmolekule. J. Biochem. (Tokyo), **5**, 133-142.

(51) Schmidt, C. L. A., and Hoagland, D. R.
1919. Table of Ph, H^+ and OH^- values corresponding to electromotive forces determined in hydrogen electrode measurements, with a bibliography. Univ. Calif. Pub. in Physiol., **5**, 23-69.

(52) Schryver, S. B.
1910. On the action of salts in heterogeneous systems, and on the nature of globulins. Proc. Roy. Soc., **83B**, 96-123, illus.

(53) Schryver, S. B., Buston, H. W., and Mukherjee, D. H.
1925. Isolation of a product of hydrolysis of proteins hitherto undescribed. Proc. Roy. Soc., **98B**, 58-65.

(54) Skraup, Z., and Hoernes, P.
1906. Über das Desamidokasein. Monatsheft, **27**, 631-652.

(55) Spiro, K., and Pemsel, W.
1898. Über Basen- und Säurecapacität des Blutes und der Eiweisskörper. Hoppe-Seyler's Z. physiol. Chem., **26**, 233-271.

(56) Takeda, M.
1922. Über die Proteinverbindungen mit Säuren oder Laugen. J. Biochem. (Tokyo), **2**, 103-115, illus.

(57) Traxl, W.
1908. Über Desamidoedestin. Monatsheft, **29**, 59-68.

(58) Van Slyke, D. D.
1911. The analysis of proteins by determination of the chemical groups characteristic of the different amino acids. J. Biol. Chem., **10**, 15-55, illus.

(59) Van Slyke, D. D., and Birchard, F. J.
1914. The nature of the free amino group in proteins. J. Biol. Chem., **16**, 539-547.

(60) Van Slyke, L. L., and Hart, E. B.
1905. Casein and paracasein in some of their relations to bases and acids. Am. Chem. J., **33**, 461-496.

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